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THE DISTRIBUTION OF THE SPORES OF B. BOTULINUS IN THE TERRITORY OF ALASKA AND THE DOMINION OF CANADA. V*

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In order to supplement the findings reported in the preceding papers, a limited number of soil specimens obtained from Alaska and the Dominion of Canada have been examined. This bacterio-geographic study serves to answer two questions: 1. Does B. botulinus occur in the soil of Alaska, and is the salmon canning industry endangered by this organism? 2. Can B. botulinus be demonstrated in the virgin forest and mountain soil of Canada?

No outbreaks of botulism due to commercially canned salmon have thus far been reported, probably on account of the fact that B. botulinus produces rapid and marked spoilage, which leads to an elimination of the abnormal tins before they reach the consumer. From a biologic and topographical standpoint, it was of interest to search for B. botulinus along the Alaskan river banks and Coast stations which house the various salmon canneries. Previous studies had shown that the rivers of the Pacific Coast slope serve as distributors of the spores from the mountains, which are the natural breeding places, so that the finding of B. botulinus, type A, in two sound clams removed from San Francisco Bay was not surprising. It was originally contemplated to study soil specimens collected at various sections of the Cordilleran system, extending along the Pacific Coast from the state of Washington to the Aleutian Islands. Unfortunately, for financial reasons, such a systematic survey was impossible, and the few samples which were procured from Alaska can only serve as guides for future investigations.

With the exception of one outbreak, reported by Glancy [1] and supposedly caused by government inspected meat or canned beets near Dawson City, Yukon, no human or animal botulism cases have been reported from Canadian territory. Samples of soil have been collected from various regions of the Dominion, and the results of the examinations form the basis for this communication.

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[1] Canadian Med. Assn. Jour., 1920, 10, p. 1027; Report 82 in the Epidemiology of Botulism, Bullet. U. S. Public Health Service, in press.

EXPERIMENTAL DATA

The methods outlined in the preceding papers have been followed in the collection and examination of the specimens. The results are herewith briefly reported.

Alaska. — Dr. E. D. Clark, Director of Pacific Fisheries Investigations, National Canners Association, Seattle, Wash., forwarded, in September, 1921, 7 samples of soil, which had been collected during the summer, at the following stations in Alaska: Alitak, Chignik River, Larsens Bay, Ikatan, Port Moller, Copper River (silt) and Copper River Bank.

The culture prepared with the samples collected at the Copper River Bank was toxic and killed 2 guinea-pigs with symptoms of botulism on the 5th and 8th day. The type could not be determined as the animals inoculated with serum invariably showed slight indefinite symptoms and recovered.

Canada.—In December, 1920, Dr. J. C. Geiger collected, in the vicinity of Vancouver, 6 samples of soil and 9 specimens of carrots, spinach, cabbage (2), greens and beets. Two samples of soils produced weak toxins.

In September, 1921, Dr. J. C. Geiger collected 20 samples of virgin soil (?) and 4 specimens of green moss along the tract of the Seymour Water supply near Vancouver, British Columbia. The cultures gave the following results:

Green moss samples, 3 B. botulinus, type A.

Virgin soil (?), 3 B. botulinus, type A, 2 weak toxins.

In October, 1921, Dr. J. C. Geiger collected 29 samples of virgin soil from the vicinity of Victoria and Lefroy Glacier, above Lake Louise, and at the foot of Mount White, Glacier, British Columbia. The cultures repeatedly gave the following results:

Red virgin soil (20), 4 B. botulinus, type A, 1 type B, 3 weak toxins.

Gray moraine and glacier soil (4), 2 B. botulinus, type B, 1 type A and B.

Black soil from Big Beehive (7,500 feet) (5), 1 B. botulinus, type A.

Dr. Henri Larouche, Chief Division of Laboratories, Montreal, submitted, in November, 1921, 16 samples of garden and road soil collected from the following counties in the Province of Quebec: St. Eustache (2); Chemin Ste. Rose-Pont Viau; Ste. Ursule, Co. Maskinonge; Pointe St. Charles, Montreal; St. Agnes de Dundee, Co. Huntingdon; Huntingdon; Bordeau; St. Cyr, Co. Richmond; Melbourne, Co. Richmond; St. Paul l'Ermite; L'Epiphanie; St. Leonard de Port Maurice; St. Michel; St. Jacques l'Achigan; Rawdon. The cultures were all nontoxic.

Dr. Frank T. Shutt, Dominion chemist, Central Experimental Station, Ottawa, kindly submitted, in December, 1921, 16 carefully collected and packed samples of virgin and manured soils procured from 8 provinces of Canada. The cultures furnished the following results:

Virgin soil, Charlottetown, Prince Edward Island, 1 B. botulinus, type A.

Manured soil, Napan, Nova Scotia, 1 B. botulinus, type A.

Virgin soil, Cape Rouge, Quebec, 1 B. botulinus, type A.

Virgin soil, Ottawa (C. Exp. Stat.), Ontario, 1 B. botulinus, type A.

Manured soil, Ottawa (C. Exp. Stat.), Ontario, 1 B. botulinus, type B.

The samples of manured soil, Charlottetown, Prince Edward Island; virgin and manured soil, Fredericton, New Brunswick; virgin soil, Nappan, Nova Scotia; manued soil, Cape Rouge, Quebec; virgin and manured soil, Brandon, Manitoba; Invermere, British Columbia, and Lethbridge, Alberta, were apparently free from spores of B. botulinus.

Summary: 61 virgin soil, 14 type A, 3 type B, 1 type A and B, 5 weak toxins; 30 cultivated or manured soil, 1 type A, 1 type B, 2 weak toxins; 9 vegetables, negative.

Total: 100 samples with 20 (27), or 20 (27.0) %, positive cultures.

The laboratory findings are summarized in table 1.

TABLE 1

SUMMARY OF LABORATORY FINDINGS

	Locality	Specimens	Total Number of Specimens Examined	Number of Toxic Cultures	Untyped Cultures	Type A	Type B	Type A and B	Percentage of Positive Samples
	ALASKA								
192	Various stations.....	Soils	7	1	1	—	—	—	—
	CANADA								
92	Vancouver, B. C.	Soils (6); vegetables (9)	15	2	2	—	—	—	—
187	Vancouver, B. C.	Soils (20); green moss (4)	24	8	2	6	—	—	25.0
200	Glacier, Lake Louise, B. C.	Soils	29	12	3	5	3	1	31.0
213	Province of Quebec....	Soils	16	—	—	—	—	—	—
215	Charlottetown, P. E. I.	Virgin and manured soil	2	1	—	1	—	—	—
	Fredericton, N. B.	Virgin and manured soil	2	—	—	—	—	—	—
	Nappan, N. S.	Virgin and manured soil	2	1	—	1	—	—	—
	Cape Rouge, Quebec...	Virgin and manured soil	2	1	—	1	—	—	—
	Ottawa, Ont.	Virgin and manured soil	2	2	—	1	1	—	—
	Brandon, Man.	Virgin and manured soil	2	0	—	—	—	—	—
	Invermere, B. C.	Virgin and manured soil	2	0	—	—	—	—	—
	Lethbridge, Alberta....	Virgin and manured soil	2	0	—	—	—	—	—
	Total: Alaska.....		7	1	1	0	0	0	
	Canada.....		100	27	7	15	4	1	
			107	28	8	15	4	1	

It has been pointed out that the number of specimens secured from Alaska is insufficient to warrant any definite conclusions. The coast soil of the Aleutian Archipelago is apparently free from spores of B. botulinus. One specimen collected from the bank of the Copper River produced on 2 occasions weakly toxic cultures which could not be typed. This river drains the Nuzotin and Wrangel Mountains, or a

part of the Cordilleran system. B. botulinus may theoretically be expected in these soil stratums, and it is most unfortunate that only 2 specimens have been collected from this region. Future studies should consider the areas along the Copper and Yukon Rivers. Tests should be made, particularly in the mountains around Mt. Allen and Mt. Kimball. Until these examinations have been made, the question whether or not B. botulinus may play a rôle in the canning of Alaskan salmon cannot be answered.

Twenty-seven, or 27%, of the 100 specimens collected in Canada furnished toxic cultures. In 20 cultures, B. botulinus, type A, as well as Type B, were repeatedly demonstrated. This anaerobe is widely distributed in the Dominion, although the Western section, namely, British Columbia and particularly the Canadian Rockies, furnishes the highest percentage of positive cultures. Attention is called to the isolation of B. botulinus, type A and type B, from glacier and mountain soil around Lake Louise. These findings fully confirm those made in the Glacier National Park and support the conception that B. botulinus is a common soil anaerobe of the Cordilleran Mountain system. B. botulinus, type A, predominated, although type B occurred occasionally either alone or in conjunction with type A. The soil samples containing type B consisted in two instances of typical moraine sand with little decaying vegetation. Without additional geologic information, it was impossible to decide whether the spores had been washed into the soil stratum or whether the organism had actually developed in this earth material. Judging by previous findings on Mount Rainier and in the Glacier National Park, it is not unlikely that the first stated view is the correct one.

Three virgin soil specimens from Prince Edward Island, Quebec, contained B. botulinus, type A, while of 2 manured soils from Nova Scotia and Ontario, 1 gave type A and 1 type B culture.

The negative results obtained with soils collected from the vicinity of Montreal and the eastern section of Quebec may be due to the selection of samples. It is, however, possible that the lower portion of the St. Lawrence River basin, draining the Great Lakes, harbors relatively few spores of B. botulinus and in this respect is comparable to and continuous with the American Great Lakes region, which is known to be slightly infected.

Sixty-one virgin soil samples furnished 23, or 37.7%, while 30 specimens from manured or cultivated fields gave only 4, or 13.3%, toxic cultures. Nine vegetable specimens were found free from B.

botulinus; 14, or 60.8%, of the toxic virgin soil specimens contained B. botulinus, type A. These findings are in accord with the observations made in the United States, and it must, therefore, be concluded that B. botulinus occurs also in Canada in an ubiquitous distribution.

CONCLUSIONS

B. botulinus has not been demonstrable in the coast land soil of the Aleutian Archipelago.

B. botulinus, type A, and occasionally type B, has been cultivated in moraine, glacier and mountain soil collected around Lake Louise in the Canadian Rockies.

Soil samples obtained from the provinces of Prince Edward Island, Nova Scotia, Quebec, Ontario and British Columbia have also furnished positive cultures.